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RESEARCH ARTICLE

Octopamine and tyramine regulate the activity of reproductive visceral muscles in the adult female blood-feeding bug, *Rhodnius prolixus*

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ABSTRACT

The role of octopamine and tyramine in regulating spontaneous contractions of reproductive tissues was examined in the female *Rhodnius prolixus*. Octopamine decreased the amplitude of spontaneous contractions of the oviducts and reduced RhoprFIRFa-induced contractions in a dose-dependent manner, whereas tyramine only reduced the RhoprFIRFa-induced contractions. Both octopamine and tyramine decreased the frequency of spontaneous bursal contractions and completely abolished the contractions at 5×10^{-7} mol l^{-1} and above. Phentolamine, an octopamine receptor antagonist, attenuated the inhibition induced by octopamine on the oviducts and the bursa. Octopamine also increased the levels of cAMP in the oviducts, and this effect was blocked by phentolamine. Dibutyryl cyclic AMP mimicked the effects of octopamine by reducing the frequency of bursal contractions, suggesting that the octopamine receptor may act by an Octβ receptor. The tyramine receptor antagonist yohimbine failed to block the inhibition of contractions induced by tyramine on the bursa, suggesting that tyramine may be acting on the Octβ receptor in the bursa.

KEY WORDS: Oviducts, Bursa, Inhibition, Contractions, Cyclic AMP

INTRODUCTION

The biogenic amine octopamine acts as a neurotransmitter, neuromodulator and neurohormone in invertebrates (Orchard, 1982). Octopamine and its precursor tyramine are both derivatives of the amino acid tyrosine, and octopamine and tyramine are believed to function analogously to adrenaline (epinephrine) and noradrenaline (norepinephrine) in vertebrates (Roeder, 2005). Thus, tyramine is now considered to be a neuroactive chemical in its own right, independent of octopamine (Kononenko et al., 2009; Lange, 2009). Octopamine and tyramine regulate diverse physiological and behavioural processes such as courtship, locomotion, learning and memory, and reproduction (Avila et al., 2012; Huang et al., 2016; Roeder, 1999; Selcho et al., 2012). Female *Drosophila melanogaster* with mutated tyrosine decarboxylase show reproductive sterility due to the lack of octopamine (Cole et al., 2005). In tyrosine decarboxylase mutant flies, supplementation with octopamine restored reproductive viability (Cole et al., 2005). Similarly, tyramine β-hydroxylase mutant flies that are found to only lack octopamine are also reproductively sterile (Monastirioti, 2003; Monastirioti et al., 1996). Octopamine and tyramine signal via G-protein coupled receptors (GPCRs), leading to changes in second messenger levels. The recently updated receptor classification (Farooqui, 2012) divides the receptors into Octα-R, Octβ-Rs (Octβ1-R, Octβ2-R, Octβ3-R), TYR1-R and TYR2-R. In general, Octβ-Rs lead to elevation of cAMP while Octα-R and TYR-Rs lead to an increase in Ca^{2+} (Farooqui, 2012).

The movement of eggs in the reproductive system of *Rhodnius prolixus* starts at the ovaries, the site of egg maturation. Upon ovulation, mature eggs are released into the oviducts (Wigglesworth, 1942). Eggs are then guided, via oviductal peristaltic and phasic contractions, to the common oviduct, where spermatozoa are released through spermathecal contractions, leading to fertilization (Davey, 1958). Fertilized eggs are coated with secretions from the cement gland (Lococo and Huebner, 1980). The bursa deposits the fertilized eggs via strong phasic contractions. These activities are under the direct control of the central nervous system (CNS) and branches of the trunk nerves innervate the reproductive tissues of *R. prolixus* (Insausti, 1994). The lateral oviducts are made up of two layers of visceral muscle, an inner circular and an outer longitudinal layer, whilst the bursa is made up of thicker muscle fibres arranged longitudinally (Sedra and Lange, 2014). The oviducts and the bursa spontaneously contract (Sedra and Lange, 2014) but the site of the intrinsic pacemaker(s) in the reproductive system has not been identified.

Octopamine and tyramine modulate the myogenic activity of a variety of visceral muscles in insects, including tissues of the reproductive system. Octopamine decreases the basal tonus, and reduces the amplitude and frequency of neurally evoked contractions of the lateral oviducts of the locust *Locusta migratoria* (Lange and Orchard, 1986). Also, octopamine has been shown to decrease the amplitude of proctolin-induced contractions in a dose-dependent manner (Lange and Orchard, 1986; Nykamp and Lange, 2000). These effects appear to be mediated by an Oct/Tyr receptor shown to be expressed in the oviducts of locusts (Molaei et al., 2005). In *Drosophila* and the stable fly *Stomoxys calcitrans*, octopamine reduces the amplitude and frequency of contractions, and reduces basal tonus of the oviducts in a dose-dependent manner (Cook and Wagner, 1992; Middleton et al., 2006; Rodríguez-Valentín et al., 2006). These physiological effects could be linked to two receptors: the octopamine receptor in the mushroom bodies (OAMB) and Octβ2-R, which have been shown in *Drosophila* to be expressed in the epithelial and muscle cells of the oviducts (Lee et al., 2003; Li et al., 2015; Lim et al., 2014). These receptors are involved in ovulation and fertilization of eggs, whereby mutant constructs of these receptors show reproductive sterility in females, accumulation of eggs in the ovary and reduction in the number of eggs laid (Lee et al., 2003; Li et al., 2015; Lim et al., 2014). In contrast, octopamine has also been shown to increase the frequency

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and the amplitude of myogenic contractions in the lateral oviducts of the cricket *Gryllus bimaculatus* (Tamashiro and Yoshino, 2014). In the cockroach *Leucophaea maderae*, the action of octopamine and tyramine is unclear; both stimulated oviduct contractions in some preparations but inhibited oviduct contractions in other preparations (Cook et al., 1984). Tyramine decreases the basal tonus and attenuates proctolin-induced contractions in locusts (Donini and Lange, 2004); however, tyramine has no effect on the amplitude of contractions or basal tonus of the oviducts in *D. melanogaster* (Middleton et al., 2006).

The purpose of this study was to determine the role of octopamine and tyramine in modulating myogenic contractions of the oviducts and the bursa of the adult female *R. prolixus* and to investigate the mechanism by which octopamine and tyramine mediate these effects.

MATERIALS AND METHODS

Animals

Adult *R. prolixus* Stål 1859 were maintained on a 12 h:12 h light:dark cycle at approximately 50% humidity and 28°C. *Rhodnius prolixus* were fed defibrinated rabbit's blood (Hemostat Laboratories, Dixon, CA, USA; supplied by Cedarlane Laboratories Inc., Burlington, ON, Canada) once in every instar. Four- to five-week-old unfed adult females were used for all experiments.

Chemicals

D,L-Octopamine hydrochloride and tyramine hydrochloride were made as 10^{-2} mol l^{-1} stocks and stored at −20°C. Phentolamine hydrochloride and dibutyryl cAMP were freshly made in physiological saline prior to use. Aliquots of AKDNFIRFamide (RhoprFIRFa, 10^{-3} mol l^{-1}; GenScript USA, Inc., Piscataqay, NJ, USA) were stored at −20°C. Stock solution of yohimbine was prepared in 95% ethanol; the final percentage of ethanol in the experimental treatments was ≤0.1%. Physiological saline (NaCl 150 mmol l^{-1}, KCl 8.6 mmol l^{-1}, $CaCl_2$ 2 mmol l^{-1}, $NaHCO_3$ 4 mmol l^{-1}, glucose 34 mmol l^{-1}, $MgCl_2$ 8.5 mmol l^{-1}, Hepes 5 mmol l^{-1}, pH 7.2) was prepared in double distilled water and used to dilute all chemicals. All chemicals were obtained from Sigma Aldrich (Oakville, ON, Canada) unless otherwise stated.

Contraction assays

Oviduct bioassay

The wings were cut off and the dorsal cuticle along with the gut of a female adult *R. prolixus* were removed to expose the reproductive system. Using a fine silk thread, a double knot was tied at the posterior end of the common oviduct and the other end of the silk was double knotted onto the hook of the force displacement signal transducer (Aksjeselskapet Mikro-elektronikk, Horten, Norway). The oviducts (lateral and common) were dissected out and placed in a Sylgard-coated dish filled with 200 µl of physiological saline at room temperature. The anterior end of each lateral oviduct was pinned to the dish with minutien pins. The signal generated was amplified, converted into a digital signal by Picoscope 2200 (Pico Technology, St Neots, UK) and analysed by the PicoLog program (Pico Technology).

To examine the effects of octopamine and tyramine on contraction of the oviducts, 100 µl of the bath saline was removed and replaced with 100 µl of 2×10^{-8} mol l^{-1} to 2×10^{-3} mol l^{-1} octopamine/tyramine. A final volume of 200 µl was maintained at all times. The tissue was washed between amine applications. For the inhibitor assays, phentolamine or yohimbine was mixed with octopamine or tyramine before application. The amplitude of three to four contractions (over ∼2 min) was averaged and presented as a percentage relative to contractions of the tissue in saline (control). To examine the effects of octopamine or tyramine on a peptide-induced contraction, 10^{-6} mol l^{-1} RhoprFIRFa was used. RhoprFIRFa produced a standard change in basal tonus which was then compared with the change in basal tonus produced when the peptide was applied with the amine.

Bursa bioassay

The dorsal cuticle was removed followed by the gut, exposing the reproductive system. Using a fine silk thread, a double knot was made at the junction of the bursa and the oviducts. A cut was made above the double knot and the bursa was left attached to the ventral cuticle and the fine silk thread was attached to the force transducer. The bursa was secured in place by pinning the ventral cuticle to the Sylgard-coated dish. The amplitude and the frequency of three to four contractions were averaged (over ∼2 min) and presented as a percentage relative to contractions of the bursa in saline (control). Amines were added to the preparations as described above for the oviduct bioassay.

cAMP determination assay

cAMP content in the oviducts of 4- to 6-week-old adult female *R. prolixus* was measured. A total of 50 oviducts were dissected and placed in a dish containing saline. Two oviducts were pooled and placed in Eppendorf tubes containing saline. Using a dispensing pipette, 10 µl of 5×10^{-3} mol l^{-1} 3-isobutyl-1-methylaxanthine (IBMX) was added to all tubes followed by the addition of either octopamine or phentolamine, or both. The tubes were gently mixed and left to incubate for 10 min. The reaction was stopped by adding 400 µl boiling ELISA buffer (Cyclic AMP ELISA Kit, Cayman Chemical, Ann Arbor, MI, USA). The tubes were boiled for 10 min and sonicated for 15 s at output 3 and constant duty cycle with a Branson Sonifier 250 (VWR, Mississauga, ON, Canada). The homogenates were centrifuged for 15 min at 13,000 rpm. Two 50 µl samples of supernatant from each tube were assayed for cAMP with the Cyclic AMP ELISA Kit (Cayman Chemical) according to the manufacturer's instructions. The pellets were dissolved in 100 µl of 1 mol l^{-1} sodium hydroxide and boiled for 10 min. The resulting solution was used for protein determination using Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis

GraphPad Prism version 5.03 (www.graphpad.com) was used to create and statistically analyse all graphs in this paper.

RESULTS

Effect of octopamine and tyramine on lateral oviduct contractions

The lateral oviducts contracted spontaneously *in vitro* as a result of the myogenic activity of the reproductive musculature (Sedra and Lange, 2014). A strong phasic contraction was initiated by one lateral oviduct and was shortly followed by contraction of the other lateral oviduct. Both oviducts then relaxed concurrently, causing a single burst (Fig. 1A,B). Twin or triple peaks in the trace were observed when the two lateral oviducts were not in sync with each other. Stable rhythmic activity was maintained for a few hours in physiological saline. Octopamine reduced the amplitude of the oviductal contractions in a dose-dependent manner (Fig. 1C). The amplitude started to decrease between 10^{-7} and 10^{-6} mol l^{-1} octopamine, with a significant decrease in amplitude at 10^{-5} mol l^{-1}

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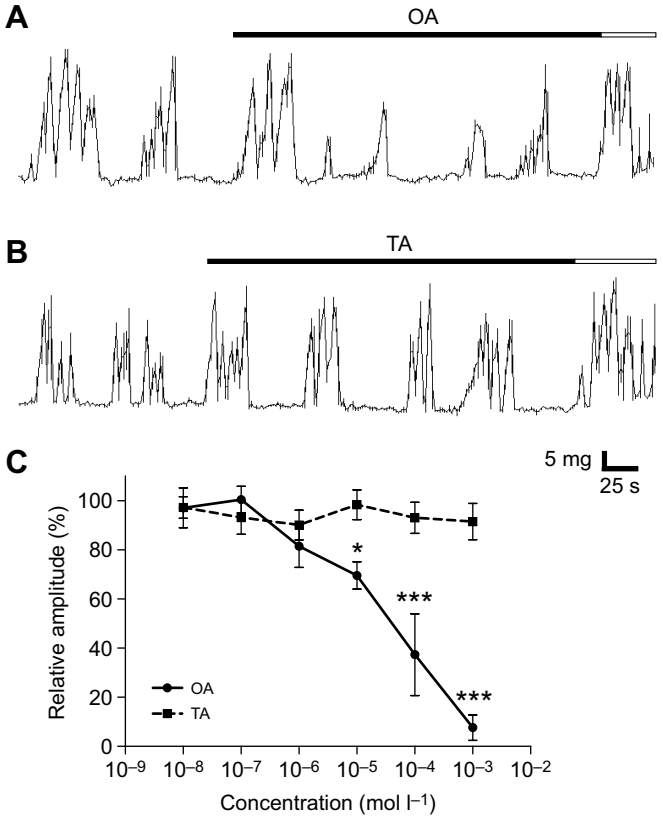

Fig. 1. The effects of octopamine and tyramine on rhythmic contractions of the oviducts of an adult female *Rhodnius prolixus*. (A) Application of octopamine (OA, 10^{-4} mol l^{-1}) inhibits of the amplitude of contractions. (B) Tyramine (TA, 10^{-4} mol l^{-1}) does not affect contractions. The black bar indicates the period of application of the neurochemical and the white bar indicates the wash period. (C) Dose–response curve for the effects of octopamine and tyramine relative to the amplitude of contractions in saline prior to the addition of neurochemicals. Octopamine inhibits the amplitude of contractions, while tyramine does not affect contraction amplitude (one-way ANOVA followed by Dunnett's multiple comparison test; *P<0.05, ***P<0.001). Data are means±s.e.m. of n=5–9 samples.

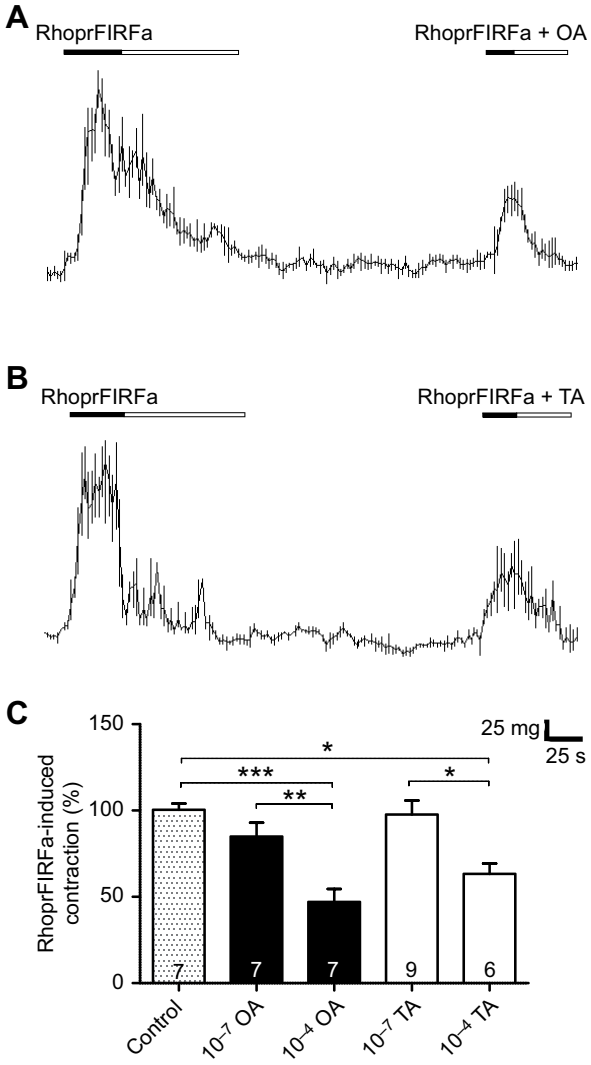

Fig. 2. Octopamine and tyramine effectively antagonize AKDNFIRFamide (RhoprFIRFa)-induced contraction of the oviducts of *R. prolixus*. (A) Octopamine (10^{-4} mol l^{-1}) significantly reduces the amplitude of the RhoprFIRFa (10^{-6} mol l^{-1})-induced contraction. (B) Tyramine (10^{-4} mol l^{-1}) significantly reduces the RhoprFIRFa (10^{-6} mol l^{-1})-induced contraction. The black bar indicates the period of application of the neurochemical and the white bar indicates the wash period. (C) Inhibition of RhoprFIRFa-induced contraction by octopamine and tyramine is dose dependent (one-way ANOVA followed by Tukey multiple comparisons test; *P<0.05, **P<0.01, ***P<0.001). Concentrations are given as mol l^{-1}; control group refers to the amplitude of 10^{-6} mol l^{-1} RhoprFIRFa-induced contraction. Means±s.e.m. of n samples noted at the bottom of each bar.

octopamine (one-way ANOVA followed by Dunnett's multiple comparison test compared with the saline group at 100%, P<0.05; Fig. 1C). At 10^{-4} mol l^{-1} and 10^{-3} mol l^{-1}, octopamine significantly reduced the rhythmic contractions by 63% and 95%, respectively, when compared with contractions in saline (one-way ANOVA followed by Tukey's multiple comparison test, P<0.001; Fig. 1A,C). Interestingly, tyramine had no effect on the amplitude of contractions (Fig. 1B,C). Tyramine at 10^{-4} mol l^{-1} did not significantly reduce the amplitude of contractions (Fig. 1B,C).

Octopamine and tyramine inhibit RhoprFIRFa-induced contractions

AKDNFIRFamide (RhoprFIRFa) is an extended FMRFamide-like peptide which belongs to the family of FMRFamide-like peptides (FLPs) (Nässel, 2002). RhoprFIRFa has been previously shown to be a strong stimulator of basal tonus of *R. prolixus* oviducts (Sedra and Lange, 2014). Application of 10^{-6} mol l^{-1} RhoprFIRFa to the oviducts produced a strong contraction that was reversible following saline wash (Fig. 2). Octopamine at 10^{-4} mol l^{-1} significantly reduced the amplitude of the RhoprFIRFa-induced contraction to 47±7.5% (one-way ANOVA followed by Tukey multiple comparisons test, P<0.001; Fig. 2A,C). Interestingly, tyramine at 10^{-4} mol l^{-1} also significantly reduced the contraction amplitude to 63±5.9% of the 10^{-6} mol l^{-1} RhoprFIRFa-induced contraction (one-way ANOVA followed by Tukey multiple comparisons test, P<0.05; Fig. 2B,C). Inhibition of the RhoprFIRFa-induced contractions was dose dependent for both octopamine and tyramine.

Octopamine and tyramine modulate bursal contractions

The bursa contracted in a rhythmic and uniform manner, producing single and defined peaks (Fig. 3). Octopamine abolished all bursal contractions at doses of 5×10^{-7} mol l^{-1} or greater (Fig. 3A,E). Lower concentrations of octopamine did not change the amplitude of rhythmic contractions (Fig. 3A,E). A similar trend was observed with tyramine, with bursal contractions abolished in all but two

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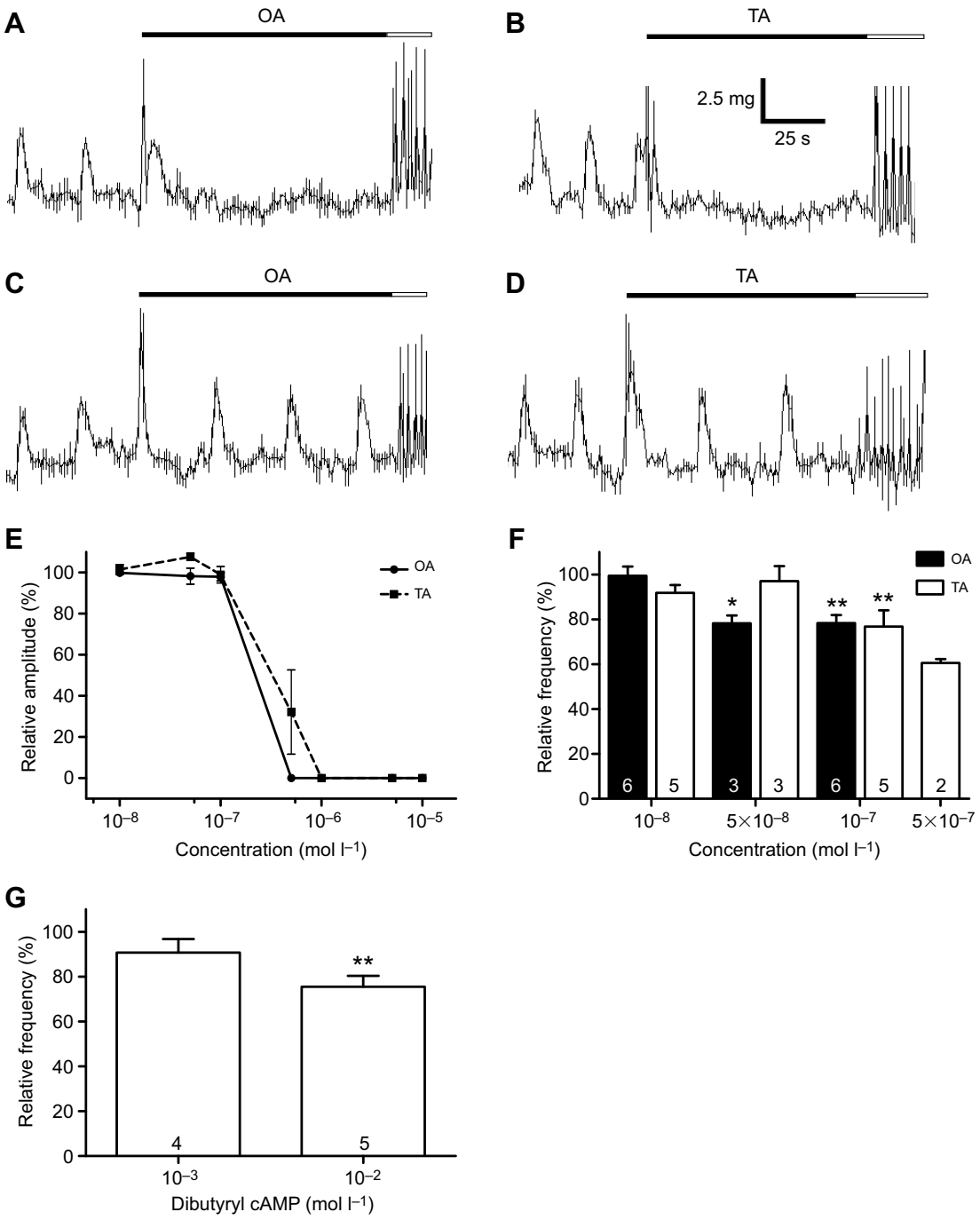

Fig. 3. Octopamine and tyramine abolish the rhythmic contractions of the bursa in *R. prolixus*. (A–D) Application of 10^{-6} mol l^{-1} octopamine (A) or tyramine (B) abolishes contractions of the bursa. Note that contractions are unchanged at 10^{-7} mol l^{-1} octopamine (C) and tyramine (D). The black bar indicates the period of application of the neurochemical and the white bar indicates the wash period. (E) Dose–response curve showing the sudden abolishment of rhythmic contractions at concentrations greater than 5×10^{-7} mol l^{-1} neurochemical (n=5–9). (F) Octopamine and tyramine both significantly decrease the burst frequency relative to the saline control. (G) Dibutyryl cAMP reduces the frequency of contractions significantly at 10^{-2} mol l^{-1} relative to the saline control. (F and G: one-way ANOVA followed by Dunnett's multiple comparison test; *P<0.05, **P<0.01.) Means±s.e.m. of n samples noted at the bottom of each bar in F and G.

preparations at 5×10^{-7} mol l^{-1} tyramine. In these two preparations, contractions were eliminated at 10^{-6} mol l^{-1} tyramine (Fig. 3B,E). Tyramine and octopamine also reduced contraction frequency of the bursa (Fig. 3F). Dibutyryl cAMP, a membrane-permeable analogue of cAMP, at 10^{-2} mol l^{-1} mimicked the effect of octopamine and tyramine in reducing the frequency of contraction to 75±4.8% relative to saline (one-way ANOVA followed by Tukey multiple comparisons test, P<0.01; Fig. 3G).

Phentolamine blocks the effects of octopamine on oviductal contractions

Phentolamine is an α-adrenergic reversible receptor blocker known to be effective at blocking OctβRs in the oviducts and skeletal muscle in locusts (Evans, 1984; Orchard and Lange, 1986). Phentolamine at 10^{-7} mol l^{-1} did not significantly alter the amplitude of oviductal contractions (one-way ANOVA followed by Tukey multiple comparisons test, P>0.05; Fig. 4C). When

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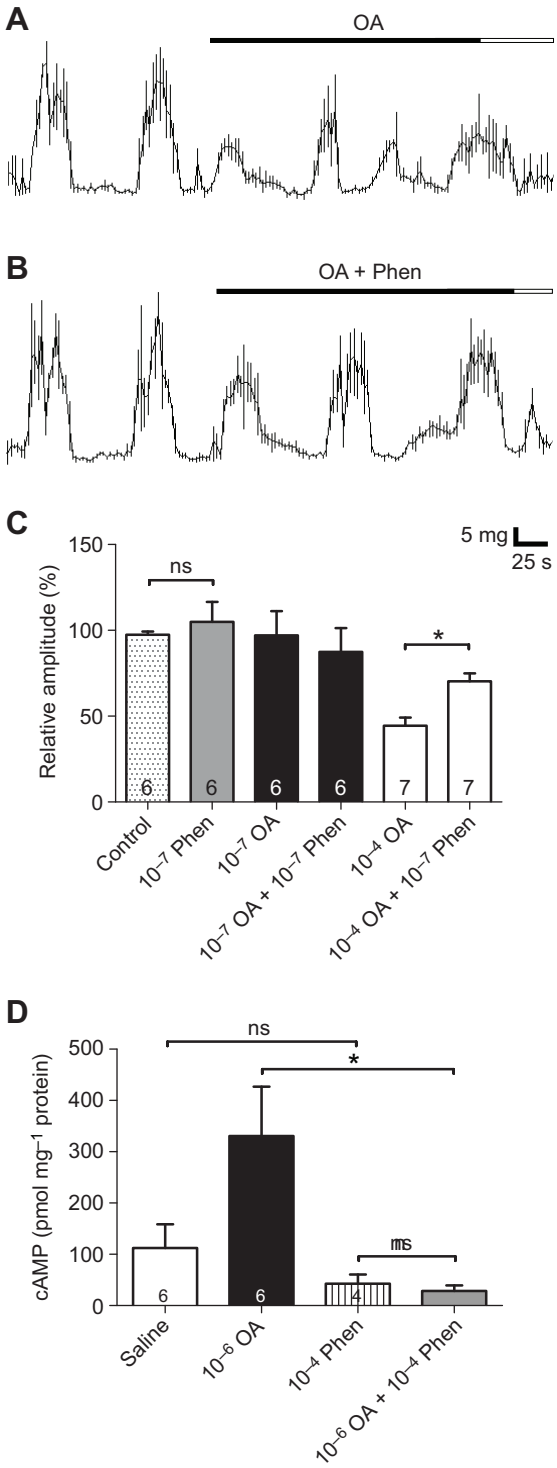

Fig. 4. Phentolamine blocks the inhibitory effect of octopamine on rhythmic contractions of the oviducts. (A) Octopamine (10^{-4} mol l^{-1}) reduces the amplitude of spontaneous contraction. (B) The effect of octopamine (10^{-4} mol l^{-1}) is inhibited by application of phentolamine (10^{-7} mol l^{-1}, Phen). The black bar indicates the period of application of the neurochemical and the white bar indicates the wash period. (C) Phentolamine alone does not affect the amplitude of contraction. Phentolamine is capable of reversing the inhibition of oviduct contraction by octopamine. (D) Phentolamine attenuates the octopamine-induced rise in cAMP levels in the oviducts. Concentrations in C and D are mol l^{-1}. (C and D: one-way ANOVA followed by Tukey multiple comparisons test; *P<0.05.) Means±s.e.m. of n samples noted at the bottom of each bar in C and D; for 10^{-6} mol l^{-1} OA+10^{-4} mol l^{-1} Phen in D, n=4.

10^{-7} mol l^{-1} phentolamine was added with 10^{-4} mol l^{-1} octopamine, the inhibition in contraction amplitude induced by octopamine was significantly reduced (one-way ANOVA followed by Tukey multiple comparisons test, P<0.05; Fig. 4). Furthermore, octopamine elevated cAMP levels in the oviducts and phentolamine at 10^{-4} mol l^{-1} attenuated this octopamine-induced increase in cAMP (one-way ANOVA followed by Tukey multiple comparisons test, P<0.05; Fig. 4D).

Phentolamine blocks the effects of octopamine on bursal contractions

Phentolamine did not significantly increase the amplitude of contraction of the bursa (one-way ANOVA followed by Tukey multiple comparisons test, P>0.05). Phentolamine at 10^{-5} mol l^{-1} significantly antagonized the effects of 10^{-5} mol l^{-1} octopamine on bursal contractions (one-way ANOVA followed by Tukey multiple comparisons test, P<0. 001; Fig. 5). Phentolamine at 10^{-5} mol l^{-1} also antagonized the effect of 10^{-5} mol l^{-1} tyramine in the bursa and restored the amplitude of contractions to 102.3±6.3% relative to the saline control.

Yohimbine does not inhibit the effects of tyramine on bursal contractions

Yohimbine is an α_2-adrenergic receptor antagonist known to block tyramine receptors (Broeck et al., 1995; Saudou et al., 1990). Yohimbine did not alter the amplitude of contractions when applied on its own and did not inhibit the effects of tyramine on bursal contractions (Fig. 6). Yohimbine at 10^{-5} mol l^{-1} failed to restore bursal contractions abolished by 10^{-5} mol l^{-1} octopamine.

DISCUSSION

Octopamine reduced the amplitude of spontaneous rhythmic contractions in *R. prolixus* oviducts. This phenomenon is consistent with results previously obtained in *L. migratoria*, *D. melanogaster* and *S. calcitrans* (Cook and Wagner, 1992; Lange and Orchard, 1986; Middleton et al., 2006; Rodríguez-Valentín et al., 2006): octopamine reduced the amplitude of contractions in *S. calcitrans* and *L. migratoria* and reduced neurally evoked contractions of *L. migratoria* oviducts (Cook and Wagner, 1992; Lange and Orchard, 1986). In *R. prolixus*, there was no change in frequency of the oviductal contractions whereas in *L. migratoria* and *S. calcitrans*, octopamine led to a decrease in basal tonus and frequency in oviduct contractions. In contrast, in *G. bimaculatus*, octopamine increased the amplitude and frequency of rhythmic contractions in a dose-dependent manner despite *L. migratoria* and *G. bimaculatus* belonging to the order Orthoptera (Tamashiro and Yoshino, 2014). In addition, octopamine decreased the amplitude of the RhoprFIRFa-induced contraction of *R. prolixus* oviducts, confirming it as an inhibitor of oviduct contractions. In locusts, it was also shown that octopamine reduces proctolin-induced contractions in the oviducts (Nykamp and Lange, 2000). Proctolin was also shown to reduce octopamine-induced cAMP levels in oviducts, suggesting that the components of octopamine and proctolin signalling pathways interact to modulate oviduct contraction (Nykamp and Lange, 2000). The interaction of RhoprFIRFa and octopamine is not known, although it is not likely that octopamine interacts with the RhoprFIRFa signalling pathway according to a recent study in *Drosophila* (Milakovic et al., 2014). Milakovic et al. (2014) found that FMRFamide-induced muscle contraction is independent of the well-known intracellular players such as calmodulin kinase II, IP_3, cAMP, etc.; however, we do not know the pathway used by RhoprFIRFa in this preparation.

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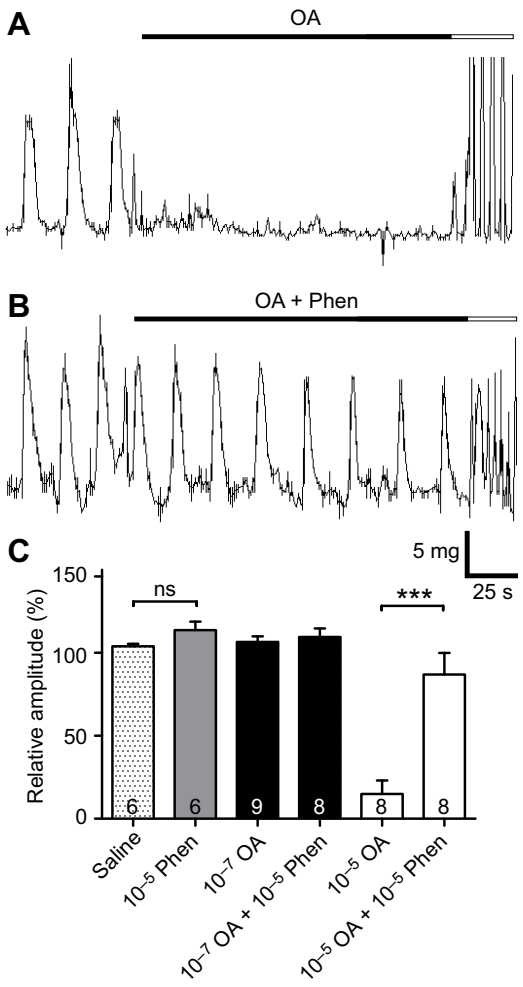

Fig. 5. Phentolamine blocks abolishment of rhythmic contractions in the bursa by octopamine. (A) Octopamine abolishes bursal contractions at 10^{-5} mol l^{-1}. (B) Phentolamine (10^{-5} mol l^{-1}) blocks the inhibition induced by octopamine (10^{-5} mol l^{-1}) on bursal contraction. The black bar indicates the period of application of the neurochemical and the white bar indicates the wash period. (C) Phentolamine at 10^{-5} mol l^{-1} does not significantly increase the amplitude of bursal contractions when compared with saline. Phentolamine significantly blocks the inhibitory effect of octopamine on bursal contractions (concentrations are mol l^{-1}; one-way ANOVA followed by Tukey multiple comparisons test; ***P<0.001). Means±s.e.m. of n samples noted at the bottom of each bar.

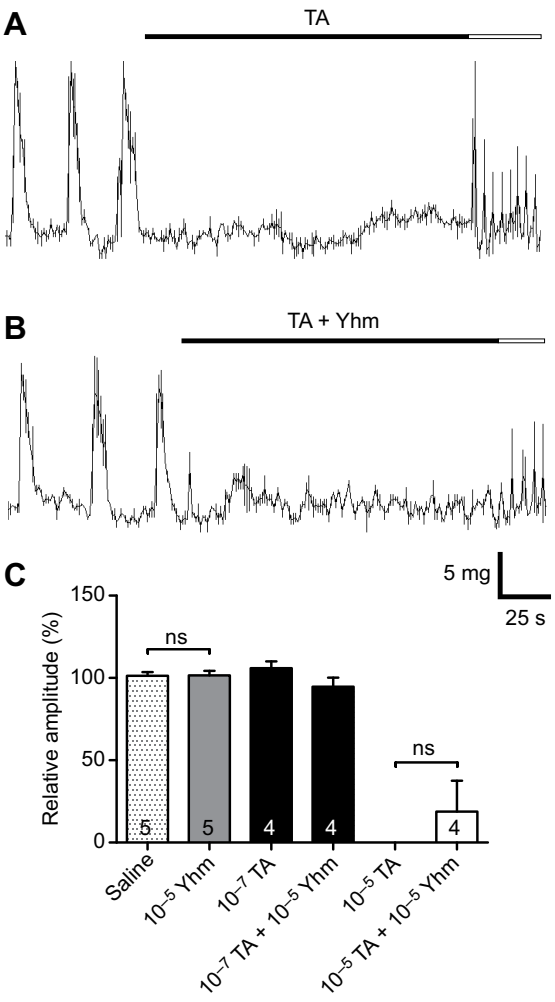

Fig. 6. Yohimbine fails to block tyramine inhibition of rhythmic contractions of the bursa. (A) Tyramine (10^{-5} mol l^{-1}) inhibits bursa contractions. (B) Yohimbine (Yhm, 10^{-5} mol l^{-1}) does not block the inhibitory effect of tyramine on bursal contractions. The black bar indicates the period of application of the neurochemical and the white bar indicates the wash period. (C) Yohimbine at 10^{-5} mol l^{-1} does not block the inhibitory effect of tyramine on the amplitude of bursal contractions (one-way ANOVA followed by Tukey multiple comparisons test; not significant, P>0.05). Concentrations are mol l^{-1}; means±s.e.m. of n samples noted at the bottom of each bar; for 10^{-5} mol l^{-1} TA, n=4.

Phentolamine, an α-adrenergic receptor antagonist, is an effective OctβR antagonist in the *L. migratoria* oviduct (Lange and Orchard, 1986; Orchard and Lange, 1986). Thus, the ability of phentolamine to block the effects of octopamine on *R. prolixus* oviducts suggests that octopamine is working via an OctβR. This is supported by the fact that octopamine increases cAMP levels (a characteristic of OctβRs) in the oviducts, an effect also blocked by phentolamine (Lange and Orchard, 1986; Orchard and Lange, 1986). The physiological implications of these findings in *R. prolixus* are that octopamine plays an essential role in the process of ovulation. Relaxation of the oviducts would allow the ovary to release more eggs into the oviducts. In *D. melanogaster*, octopamine was found to increase the contractions of the peritoneal sheath, a contractile meshwork that surrounds the ovary, and to relax the oviducts, thereby enabling the release of eggs into the oviducts (Middleton et al., 2006). Moreover, Octβ2R and OAMB have been found to be the receptors associated with the process of ovulation and fertilization in *Drosophila* (Lee et al., 2003; Li et al., 2015; Lim et al., 2014). Deletions in the *oamb* locus and mutant constructs of Octβ2R resulted in accumulation of eggs in the ovary and a significant decrease in the number of eggs laid (Lee et al., 2003; Li et al., 2015; Lim et al., 2014). Similarly, in *R. prolixus*, octopamine reduced the amplitude of oviduct contractions, probably by binding to OctβR, leading to an elevation in cAMP levels and muscle relaxation.

The process by which tyramine regulates the oviducts seems more modulatory. Tyramine, when applied to the oviducts at a wide range of doses did not elicit any changes in spontaneous contractions; however, tyramine inhibited RhoprFIRFa-induced contractions in a dose-dependent manner. This is not the case in *L. migratoria*, where tyramine mimicked octopamine and decreased the basal tonus and attenuated proctolin-induced contractions (Donini and Lange, 2004). A possible explanation for this phenomena in *R. prolixus* is that tyramine is co-released with octopamine; octopamine works on the oviducts to reduce the amplitude of spontaneous contractions induced by a pacemaker,

whereas octopamine and tyramine modify the effects of myogenic stimulators such as RhoprFIRFa.

The effects of octopamine and tyramine on the bursa are similar. Both biogenic amines completely abolish contractions of the bursa at high concentrations. In addition, octopamine and tyramine at low concentrations decrease the frequency of contractions. These effects seem to be mediated by cAMP, as application of the membrane-permeable cAMP analogue dibutyryl cAMP decreased the frequency of contractions. Phentolamine antagonized the effects of octopamine and tyramine, suggesting that both are likely to work via an OctβR. Yohimbine did not antagonize the effects of tyramine and octopamine on the bursa. This suggests that tyramine acts via the OctβR at high concentrations, as shown in the locust oviducts and foregut (Britain, 1990; Donini and Lange, 2004). Further studies are needed to understand why the contractions are abolished with no apparent effect on the amplitude of contraction. Octopamine and tyramine may inhibit the pacemaker activity at the bursa. cAMP is the likely second messenger involved in this process as similar results were seen when dibutyryl cAMP was applied. In vertebrate models, cAMP can directly modify the activity of ion channels in pacemaker cells to increase or decrease myogenic activity, thereby altering the timing of activity (Wainger et al., 2001).

This study reveals that octopamine and tyramine lead to the relaxation of the oviducts, aiding in the process of ovulation. In the bursa, the biogenic amines may lead to egg retention by attenuating bursal contractions and preventing oviposition. Octopamine and tyramine probably work with other neurotransmitters and hormones to control processes to ensure the laying of viable eggs.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization, A.B.L. and S.H.; Methodology, A.B.L. and S.H.; Investigation, S.H.; Writing – Original Draft, S.H.; Writing – Review & Editing, A.B.L. and S.H.; Funding Acquisition, A.B.L. and S.Y.W.; Supervision, A.B.L.

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